EXHIBIT A83

ELSEVIER

Contraception

Contraception 63 (2001) 41–45
Original research article

Evaluation of active and passive transport mechanisms in genital tracts of IUD-bearing women with radionuclide hysterosalpingoscintigraphy

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Received 27 July 2000; revised 16 November 2000; accepted 20 November 2000

Abstract

The objective of this study was to evaluate the active and passive transport mechanisms in the genital tracts of copper T-200 intrauterine device (IUD)-bearing women. $^{Tc\text{-}99m}$HMPAO-labeled spermatozoa and $^{Tc\text{-}99m}$-labeled albumin macrospheres were placed into the vagina at midcycle. Serial scintigraphic images were obtained over a period of 2 h. Migration of spermatozoa and particles in the genital tract and the direction of transport related to dominant follicle were evaluated. While active sperm migration was greatly inhibited, the passive transport of the particles was not affected in IUD-bearing women. The direction of radiolabeled particles and spermatozoa was toward the dominant follicle side. Passive transport was not affected, whereas active transport of spermatozoa was strongly inhibited in the genital tract by the presence of the IUD. However, the direction of active and passive transport related to dominant follicle side was unchanged in IUD-bearing women and was preferentially toward the tube ipsilateral to the dominant follicle. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Intrauterine devices; Radionuclide hysterosalpingography; Tubal investigation; Sperm transport; Female genital tract

1. Introduction

Intrauterine devices (IUDs) have become widely used since their introduction, but their specific contraceptive action is far from clear. The mode of action of the IUD is important, since if the IUD is an abortifacient, it could have important moral, religious, and ethical repercussions. However, it is generally believed that all IUDs stimulate an inflammatory response in the uterus [1,2]. Many studies have reported that numerous polymorphonuclear leukocytes appear in the endometrium of IUD users followed by foreign body giant cells, mononuclear cells, and macrophages [3]. In women using an IUD, sensitive assays for human chronic gonadotropin provide evidence of implantation in $<$1% of menstrual cycles [4,5].

Regarding migration patterns of spermatozoa from the vagina to the upper segments of the genital tract and the peritoneal cavity, either in the presence or absence of an IUD, are scarce and contradictory. The effect of an IUD on the early phase of sperm migration can be estimated by comparing the results of Settlage et al. [6] who described sperm migration in non-IUD users, with those of Tredway et al. [7] who studied this process in IUD users. Both studies, done by the same group of investigators, searched for spermatozoa in the flushing of excised tubes 15–30 min after insemination. Spermatozoa were present in the oviducts of all control women not using IUD, while no spermatozoa were found in the tubes of IUD users. Other investigators have also reported decreased numbers or absence of spermatozoa in IUD users [8,9]. However, the evidence derived from these and similar studies are limited because of possible artifacts introduced by invasive techniques such as anesthesia and laparotomy.

In the present study, sperm migration was first assessed by radiolabeled sperm in IUD-bearing women. Serial hysterosalpingoscintigraphy (HSSG) was performed during the ovulation phase of the menstrual cycle to obtain insights into the mechanisms that govern sperm migration in the genital tract of IUD-bearing women. We also evaluated the direction of sperm transport toward the right or left tube with respect to the side of ovulation in IUD-bearing women. Furthermore, HSSG allows us to compare the passive trans-

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PII: S0010-7824(00)00190-6

port process (using technetium-^{99m}-labeled-albumin macro spheres) with active transport process (using technetium-^{99m} hexamethylpropylene amine oxime labeled-spermatozoa) in the genital tract of IUD-bearing women.

2. Materials and methods

A total of 34 women between the ages of 22–38 years (mean ± SD; 31.9 ± 4.2) were included in this study after giving informed consent. The study was approved by the local ethics committee. All subjects had regular cycles, and were without a history of infertility or current pelvic infection. The investigation took place at mid-cycle (possibly at ovulation) as evidenced by appropriate serum estradiol and luteinizing hormone levels, cervical mucus spinnbarkeit greater than 8 cm, and preovulatory ovarian follicles at vaginal ultrasonography. The dominant follicle side was also determined. Ovulation was proved by levels of progesterone of >5 ng/mL at 21st days of menstrual cycle in all subjects. Women refrained from sexual intercourse for at least 2 days before the study.

The IUD users group (group 1) consisted of 14 women between the ages of 22 and 38 years (mean ± SD; 28.2 ± 3.6) who had been using the copper T-200 IUD for at least 12 months. The second group was composed of 10 women, 19 to 29 years of age (mean ± SD; 24.3 ± 3.7), who had also been using the copper T-200 IUD for at least 12 months. The tubal ligation group (group 3) was composed of 10 women, 29 to 37 years of age (mean ± SD; 32.4 ± 4.3), who had undergone laparoscopic proximal tubal surgical sterilization between 3 months and 4 years previously.

The spermatozoa were radiolabeled using the method described by Ozgur et al. [10]. The husbands' semen samples were collected by masturbation and kept in sterile petri dishes at 25°C for 20 min to allow for liquefication. Sperm concentration and sperm motility were determined with a hemocytometer to ensure an adequate number of motile cells. One mL of semen was mixed with 3 mL of the medium (Ham's F10 solution, 320-1550 AG, GIBCO BRL Life Technologies, Gaithersburg, MD, USA) 2 to 3 times greater in volume and centrifuged at 1500–2000 rpm for 10 min. The supernatant was discharged and the pellet was resuspended in 2 mL of the medium and recentrifuged at the same speed and time. $^{Tc\text{-}99m}$HMPAO (Technetium 99m hexamethylpropylene amine oxime, "Frederic Joliot-Curie" National Research Institute for Radio-biology and Radiohygiene, Budapest, Hungary) with a total activity of 9.3 to 11.2 MBq (0.18 mg/0.5 mL) was added to the pellet and the cells were incubated for 30 min at 37°C in a water bath. The radio labeled sample was recentrifuged at 1500–2000 rpm for 10 min. and the supernatant was discarded. The pellet was resuspended in 1 mL of Ham's F10 and reassessed for sperm motility.

HSSG examination was performed with radiolabeled albumin macrospheres (MAA, Mallincrodt Medical, Petten, Holland) in group 3 according to the method described by Iturralde and Venter, and Becker et al., with some modifications [11,12]. Albumin macrospheres with a 95% mean diameter of 10–90 μm were labeled with Tc-99m and suspended with normal saline 5 min prior to application; thus, 1 mL of the suspension containing 0.5×10^6 labeled albumin particles with a radioactivity of approximately 18.5 MBq was prepared.

The women were placed in the supine gynecological examination position. The uterine cervix and posterior vaginal fornix were exposed. $^{Tc\text{-}99m}$HMPAO-labeled spermatozoa and $^{Tc\text{-}99m}$-labeled albumin macrospheres were placed by a syringe into the posterior vaginal fornix while the patient was in the supine position in Groups 1, 2, and 3. The position of the patients was not changed during the entire scintigraphic procedure.

Serial anterior scintigraphic imagines were obtained using a gamma camera (General Electric 3200 × R/T) over a period of 2 h. During the first 10 min the radioactivity was measured every minute, and thereafter at every 5 min for 1 h and at 75, 90, and 120 min. The distribution of radioactivity after discharging of spermatozoa or MAA into the vaginal fornix toward fallopian tubes through the uterine cavity was evaluated by showing the relative distribution of radioactivity. Migration of sperm and particles was determined independently by two nuclear medicine specialist and a gynecologist who did not know the patients' group. The appearance of radioactivity adjacent to the uterus was considered evidence of tubal transport for that side. The lack of distribution of radioactivity beyond the uterus was considered as an absence of tubal transport. The relationship between the ovulation side and direction of transport was also evaluated. Statistical analysis were performed using Student's *t* test and Spearman rank correlation analysis, were appropriate.

3. Results

Migration of radiolabeled sperm and MAA was observed in the genital tract of all women. A band of activity was seen at the vaginal fornices. The uterine cavity could be identified after 2 to 5 min. The uterine activity tended to vary in position and shape during the course of examination, which was attributed to changes in the uterine position.

Radiolabeled sperm did not migrate into the fallopian tubes in 9 of 14 (65%) IUD-bearing women (Fig. 1) and left or right tubal migration of radiolabeled spermatozoa was detected in 5 of 14 (35%) IUD-bearing women of group 1. The ascension of the radioactivity was mainly directed into the tube ipsilateral to the dominant follicle in all of these 5 subjects. As judged from the prints shown in Fig. 2, no radioactivity was ever found in the contralateral tube.

Tubes were considered as occluded if the activity ascended to the uterus, and remained constant during the

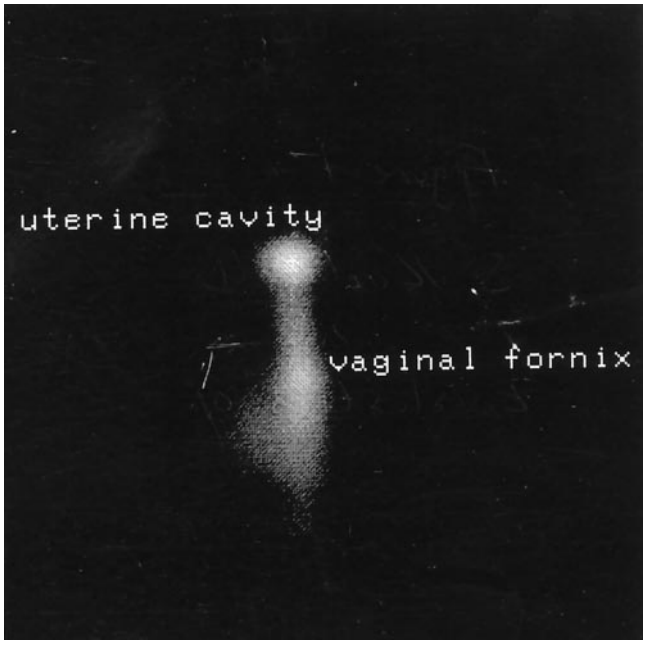

Fig. 1. HSSG showing blockage of radiolabeled spermatozoa into the uterine cavity.

entire scanning period without progression to the tubes. As expected, bilateral tubal occlusions were detected in all women of group 3 who had undergone bilateral tubal ligation. The absence of radiolabeled spermatozoa in the fallopian tubes in all subjects of bilateral tubal ligation group (group 3) was evidence of the effectiveness of the HSSG method in evaluating the transport mechanisms.

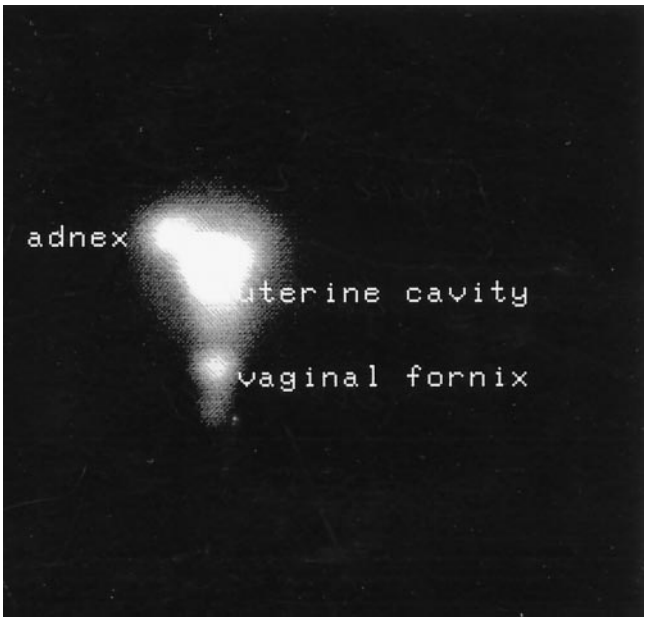

Fig. 2. HSSG showing the ascension of radiolabeled spermatozoa into the tube ipsilateral to dominant follicle, note the lack of radioactivity into the contralateral tube.

Ascension of labeled particles from the vaginal fornix, to the uterine cavity, into the fallopian tubes was shown in all IUD-bearing women in group 2. The direction of the radiolabeled particles was toward the dominant follicle side in all subjects (10 of 10, 100%) of group 2. This means that passive transport and the direction of the particles related to the dominant follicle were not changed in IUD-bearing women.

The ascension of radiolabeled spermatozoa and MAA was toward the dominant follicle side in 5 of 5 subjects and 10 of 10 subjects in Groups 1 and 2, respectively. The correlation between the direction of radioactivity and ipsilateral dominant follicle was statistically significant in Groups 1 and 2 ($p < 0.01$).

The comparison of the transport of radiolabeled spermatozoa with radiolabeled particles toward the fallopian tubes in IUD-bearing women revealed that while active sperm migration were greatly inhibited (9 of 14 subjects, 65%), passive transport of the particles was not affected (10 of 10 subjects, 100%) in IUD-bearing women ($p < 0.01$). The direction of active and passive transport related to the dominant follicle was not changed in IUD-bearing women.

4. Discussion

The mode of action of the intrauterine device as a contraceptive agent has not been established. The theory concerning its mechanism of action is an alteration in myometrial or endometrial milieu resulting in an effect on gamete transport.

Tredway et al. [7] examined the presence of sperm in oviducts after vaginal insemination in IUD-bearing women who were having bilateral salpingectomies for sterilization. In their study, no sperm was found in the oviducts of any subjects by histologic examination. The results of this study indicate that the IUD interferes with sperm transport in the human.

Segal et al. [4] measured serum progesterone, luteinizing hormone and human chorionic gonadotropin levels in IUD users, women with tubal ligation, and women trying to become pregnant. They concluded that IUD users do not retain their natural fertility, and that the IUD does not exert its antifertility effect as an abortifacient [4].

To gain a better understanding of the mechanism of IUD action, Alvarez et al. searched for ova and embryo in the genital tract of 115 women using no contraception and of 56 women using IUDs. Ova and embryos were recovered from the tubal flushing between 48 and 120 h after the mid cycle LH peak. No fertilized ova were recovered from the body of the uterus of any of the IUD users [13].

Failure of fertilization resulting from the presence of an IUD may be the result of spermatozoa blockage in the uterine cavity. Comparative studies on the number and

condition of spermatozoa recovered from the tubes of women using IUDs versus those without IUDs are contradictory. Some studies show no difference [14,15], while others report either a decrease in the number or an absence [7,16] of spermatozoa in the presence of IUDs. All of these studies were depend on histologic or biochemical markers of early embryo or gametes.

The present study differs from previous studies in several ways. First, is the HSSG method, which was used to assess the effectiveness of IUD. The HSSG method is more physiologic, objective and less invasive than the previous techniques. By using HSSG, we can exclude the artifacts due to anesthesia and/or laparotomy. Biochemical and hormonal methods are indirect markers of fertilization and gamete transport; so they could not give any information about the sperm transport mechanisms in IUD users. Contractions of the reproductive tract would be anticipated in the presence of prostaglandins. In this study, sperm were washed; thus the effects of prostaglandins were not included in the study design (Kadanali, personal observation).

Tubal fluid is stimulated by estrogen at midcycle, as well as the protein content and muscular contraction of the tube also peaks at the periovulatuar period [17], we have to study the probable fertilization period to gain a better understanding of the antifertility effects of IUDs. As it others, this study has ethical limitations, it would be impossible to have a positive control group where radiolabeled spermatozoa were administered to non-IUD users at the time of ovulation to compare the fertility results with IUD users. We tried to minimize this ethical limitation by including a negative control group consisting of women who had undergone tubal ligation (group 3) and IUD-bearing subjects whom passive transport of the radiolabeled particles was evaluated (group 2).

According to our data the following concept on the dynamics of sperm transportation within the female genital tract in IUD-bearing women may be proposed: sperm transportation from vaginal fornix to the fallopian tubes is generally inhibited (65%) during the time of ovulation in IUD-bearing women.

The most striking finding in our study is the preferential direction of sperm transport and particles into the tube ipsilateral to the dominant follicle in IUD-bearing women. Although the preferential direction of particles and spermatozoa toward the ipsilateral to the dominant follicle was recently shown by others [17,18], this study was first showing the preferential transport is also true for IUD-bearing women, so that the IUD does not have an effect on the preferential transport of particles and spermatozoa into the tube ipsilateral to dominant follicle.

The finding of 65% inhibition rate of spermatozoal transport into the tubes in IUD-bearing women suggested that there might be an endometrial blockage against the spermatozoa in IUD-bearing women. Previous and recent studies proposed a change in endometrial milieu and a defective synthesis of some endometrial proteins such as PP12, PP14 in IUD-bearing women [19].

The other interesting finding of this study is that while active sperm transport was greatly inhibited (9 of 14 IUD-bearing woman), the passive transport of particles was not affected (10 of 10) in IUD-bearing women. We do not have any information to explain this data, but there appears to be a biologic or histologic barrier against spermatozoa, but not for MAA particles. It is believed that uterine contractions aspirate spermatozoa into the cervical mucus and the uterine cavity, and provide further transport into the isthmus; this transport is directed into the tube ipsilateral to the dominant follicle. This indicates that mechanisms of rapid and passive transport are under the endocrine control of the dominant follicle. On the basis of data obtained here, we postulate that the passive transport was not affected by the presence of the IUD, whereas active transport of spermatozoa was strongly inhibited by the presence of IUD. This may be an important factor in the effectiveness of IUDs. Contraception due to IUD might be greatly regulated by the blockage of spermatozoal transport.

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